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(57) Abstract

The present invention provides a chimeric polypeptide comprising a hepatitis B virus core antigen (HBcAg) region and a region comprising an epitope of a tumor antigen. The present invention further provides a nucleic acid encoding the chimeric polypeptide, a vector comprising the nucleic acid and a cell comprising the vector. Also provided is a method of inhibiting the growth of tumor cells in a subject, comprising administering to the subject a tumor cell growth-inhibiting amount of a chimeric polypeptide comprising a HBcAg region and a region comprising an epitope of a tumor antigen corresponding to a tumor antigen in the subject. A chimeric polypeptide comprising a hepatitis B virus core antigen region and an antigenic polypeptide region comprising greater than 40 amino acids, which assembles into core particles, is also provided, as well as a method for constructing a chimeric polypeptide comprising a hepatitis B virus core antigen region and an antigenic polypeptide region comprising greater than 40 amino acids, which assembles into core particles, comprising cloning into an expression vector a first DNA fragment encoding the hepatitis B core antigen region and a second DNA fragment encoding an antigenic polypeptide region greater than 40 amino acids; and expressing the DNA of the expression vector in an expression system whereby the chimeric polypeptide assembles into core particles.

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METHODS AND COMPOSITIONS OF CHIMERIC POLYPEPTIDES FOR TUMOR ANTIGEN VACCINES

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to a vaccine that elicits an immune response against tumors. In particular, the present invention provides a chimeric polypeptide comprising an epitope of hepatitis B virus core antigen (HBcAg) and an epitope of a tumor antigen, as well as methods for administering the chimeric polypeptide to a subject to inhibit tumor growth.

BACKGROUND ART

Tumor cells are known to express tumor-specific antigens on the cell surface. These antigens are believed to be poorly immunogenic, largely because they represent gene products of oncogenes or other cellular genes which are normally present in the host and are therefore not clearly recognized as nonself. Although numerous investigators have tried to target immune responses against epitopes from various tumor specific antigens, none have been successful in eliciting adequate tumor immunity *in vivo* (for review, see, for example, Finn et al., 1995. *Immunological Reviews* 145:61-88; "Partially purified tumor antigen vaccines" (section 23.4), In: *Biologic Therapy of Cancer: Principles and Practice*, 2nd ed. Edited by DeVita et al., J.B. Lippincott Co., 1995).

Humans are particularly vulnerable to cancer as a result of an ineffective immunogenic response. (Old, L.J. 1981. "Cancer immunology: The search for specificity," GHA Clowes Memorial Lecture. *Cancer Res.* 41:361-375). In fact, the poor immunogenicity of relevant cancer antigens has proven to be the single greatest obstacle to successful immunotherapy with tumor vaccines. (Livingston, P. In: *Biologic*

Therapy of Cancer: Principles and Practice, 2nd ed. Edited by DeVita et al., J.B. Lippincott Co., 1995). Over the past 30 years, literally thousands of patients have been administered tumor cell antigens as vaccine preparations, but the results of these trials have demonstrated that tumor cell immunization has failed to provide a rational basis for the design or construction of effective vaccines. Even where patients express tumor-specific antibodies or cytotoxic T-cells, this immune response does not correlate with a suppression of the associated disease. This failure of the immune system to protect the host may be due to expression of tumor antigens that are poorly immunogenic or to heterologous expression of specific antigens by various tumor cells. The appropriate presentation of tumor antigens in order to elicit an immune response effective in inhibiting tumor growth remains a central issue in the development of an effective cancer vaccine.

Nucleocapsid or core antigen from hepatitis B virus (HBV) is known to be highly immunogenic and to have very efficient T-cell helper function. The protein subunit spontaneously assembles into 27 nm core particles when expressed in *Escherichia coli*. HBcAg has been used as a carrier of peptide epitopes from viral and bacterial proteins linked by genetic engineering to yield a specific immune response to these epitopes. In one study, a chimeric polypeptide comprising several human papillomavirus (HPV) B-epitopes and a "universal" T-helper epitope linked to HBcAg was found to elicit antibody and T-helper responses in mice (Tindle et al. 1994. *Virology* 200:547-557). Such results were not surprising because the epitopes were selected because they were already known to be significantly immunogenic by themselves. The combination of HBcAg with poorly immunogenic antigens, such as autologous tumor antigens, as vaccine candidates has not previously been examined. In addition, the fusion of HBcAg to antigenic polypeptides greater than 40 amino acids has not previously been described (Borisova et al., 1993. *J. Virol.* 67:3696-3701).

Therefore, there remains a great need for a method of presenting autologous tumor antigens, which are known to be poorly immunogenic, "self" antigens to a subject's immune system in a manner that elicits an immune response powerful enough to inhibit the growth of tumor cells in the subject. This invention overcomes the previous limitations and shortcomings in the art by providing a chimeric polypeptide comprising HBcAg and an epitope of a tumor antigen which can produce an *in vivo* immune response resulting in the inhibition of tumor cells. The present invention also greatly improves the versatility of the use of fusion proteins containing HBcAg by providing a chimeric polypeptide comprising HBcAg and an antigenic polypeptide region comprising greater than 40 amino acids.

SUMMARY OF THE INVENTION

The present invention provides a chimeric polypeptide comprising a hepatitis B virus core antigen (HBcAg) region and a region comprising an epitope of a tumor antigen. The present invention further provides a nucleic acid encoding the chimeric polypeptide, a vector comprising the nucleic acid and a cell comprising the vector.

Also provided is a method of inhibiting the growth of tumor cells in a subject, comprising administering to the subject a tumor cell growth-inhibiting amount of a chimeric polypeptide comprising a HBcAg region and a region comprising an epitope of a tumor antigen corresponding to a tumor antigen in the subject.

The present invention further provides a chimeric polypeptide comprising a HBcAg region and an antigenic polypeptide region comprising greater than 40 amino acids.

Furthermore, the present invention provides a chimeric polypeptide comprising a HBcAg region and a antigenic polypeptide region comprising greater than 40 amino acids, which assembles into core particles.

Also provided is a method of constructing a chimeric polypeptide comprising a HBcAg region and an antigenic polypeptide region comprising greater than 40 amino acids, which assembles into core particles, comprising cloning into an expression vector a first DNA fragment encoding a HBcAg region and a second DNA fragment encoding an antigenic polypeptide region greater than 40 amino acids; and expressing the DNA of the expression vector in an expression system whereby the chimeric polypeptide assembles into core particles.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

As used in the claims, "a" can include multiples.

The present invention provides a chimeric polypeptide comprising a HBcAg region and a region comprising an epitope of a tumor antigen. The chimeric polypeptide can be present in a purified form and can induce an immune response against the epitope of the tumor antigen and inhibit the growth of tumor cells expressing the epitope of the tumor antigen. "Purified" as used herein means the polypeptide is sufficiently free of contaminants or cell components with which proteins normally occur to allow the peptide to be used therapeutically. It is not contemplated that "purified" necessitates having a preparation that is technically totally pure (homogeneous), but purified as used herein means the chimeric polypeptide is sufficiently pure to provide the polypeptide in a state where it can be used therapeutically. As used herein, "chimeric polypeptide" means a polypeptide made up of two or more amino acid sequences representing peptides or polypeptides from different sources. Also as used herein, "epitope" refers to a specific amino acid sequence of limited length which, when present in the proper

conformation, provides a reactive site for an antibody or T cell receptor. The identification of epitopes on antigens can be carried out by immunology protocols that are standard in the art, such as, for example, as set forth in Harlow & Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1988. As further used herein, "tumor antigen" describes a polypeptide expressed on the cell surfaces of specific tumor cells and which can serve to identify the type of tumor. The epitope of the tumor antigen can be any site on the antigen that is reactive with an antibody or T cell receptor. The tumor antigen can be from any type of tumor now known or identified in the future.

The appropriate tumor antigen used in the chimeric polypeptide naturally depends on the tumor type being treated. For example, the tumor antigen can be, but is not limited to human epithelial cell mucin (MUC1), the Ha-ras oncogene product, p53, carcino-embryonic antigen (CEA), the raf oncogene product, GD2, GD3, GM2, TF, sTn, MAGE-1, MAGE-3, tyrosinase, gp75, Melan-A/Mart-1, gp100, HER2/neu, EBV-LMP 1 & 2, HPV-F4, 6, 7, prostatic serum antigen (PSA), alpha-fetoprotein (AFP), CO17-1A, GA733, gp72 and any other tumor antigens now known or identified in the future. The effectiveness of the chimeric protein in eliciting an immune response against a particular tumor antigen can be determined following the methods set forth in the Examples.

Tumor antigens can be obtained following known procedures or are commercially available. See generally, The Oncogene Handbook, T. Curran, E.P. Reddy, and A. Salka (ed.), Elsevier Science Publishers, The Netherlands (1988).

The HBcAg region of the chimeric polypeptide can comprise the HBcAg protein in its entirety or a portion of the HBcAg protein. Thus, sections of the HBcAg gene containing the amino terminus, sections of the HBcAg gene containing the carboxy terminus and/or sections of the HBcAg gene encoding internal amino acid sequences can

be cloned separately or in any combination into the construct of the chimeric polypeptide, according to the teachings herein. A section of interest can initially be chosen on the basis of its potential for immunogenicity as indicated by computer models and mutation analysis and its suitability as part of the chimeric polypeptide of the present invention can be determined by measuring the immunogenic and therapeutic response of the section of interest, linked to an epitope of a tumor antigen, according to standard techniques in the art, such as, for example, as taught herein in the Examples.

As will be appreciated by those skilled in the art, the invention also includes peptides and polypeptides having slight variations in amino acid sequences or other properties. Such variations may arise naturally as allelic variations (*e.g.*, due to genetic polymorphism) or may be produced by human intervention (*e.g.*, by mutagenesis of cloned DNA sequences), such as induced point, deletion, insertion and substitution mutants. Minor changes in amino acid sequence are generally preferred, such as conservative amino acid replacements, small internal deletions or insertions, and additions or deletions at the ends of the molecules. Substitutions may be designed based on, for example, the model of Dayhoff, *et al.* (in *Atlas of Protein Sequence and Structure 1978*, Nat'l Biomed. Res. Found., Washington, D.C.). These modifications can result in changes in the amino acid sequence, provide silent mutations, modify a restriction site, or provide other specific mutations. The polypeptides can comprise one or more selected epitopes on the same tumor antigen, one or more selected epitopes on different tumor antigens, as well as repeats of the same epitope, either in tandem or interspersed along the amino acid sequence of the HBcAg polypeptide. The epitope of the tumor antigen can be positioned in the chimeric polypeptide at the carboxy terminus of HBcAg, the amino terminus of HBcAg and/or at one or more internal sites within the HBcAg amino acid sequence.

Also provided is a composition comprising a chimeric polypeptide comprising a HBcAg region and a region comprising an epitope of a tumor antigen, and a

pharmaceutically acceptable carrier. The composition can include an effective amount of the chimeric polypeptide in combination with a pharmaceutically acceptable carrier and in addition, can include other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, etc. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected antigen without causing substantial deleterious biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences (Martin, E.W., ed., latest edition, Mack Publishing Co., Easton, PA).

In a further preferred embodiment, the composition can comprise a suitable adjuvant. As used herein, "suitable adjuvant" describes an adjuvant capable of being combined with the chimeric polypeptide to enhance an immune response in a subject without deleterious effect on the subject. A suitable adjuvant can be, but is not limited to, for example, SYNTEX adjuvant formulation 1 (SAF-1) composed of 5 percent (wt/vol) squalene (DASF, Parsippany, N.J.), 2.5 percent Pluronic, L121 polymer (Aldrich Chemical, Milwaukee), and 0.2 percent polysorbate (Tween 80, Sigma) in phosphate-buffered saline. Other suitable adjuvants are well known in the art and include Freund's adjuvant, alum, aluminum phosphate, aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trealose dimycolate and cell wall skeleton (MPL+TDM+CWS) in 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the immune response

directed against the chimeric polypeptide using standard procedures, as described herein.

An isolated nucleic acid encoding the chimeric polypeptides as described above is also provided. By "isolated nucleic acid" is meant nucleic acid molecules that are substantially free of the other nucleic acids and other components commonly found in association with nucleic acid in a cellular environment. Separation techniques for isolating nucleic acids from cells are well known in the art and include phenol extraction followed by ethanol precipitation and rapid solubilization of cells by organic solvent or detergents (See, e.g., *Sambrook et al.*, 1989. *Molecular Cloning: A Laboratory Manual*, 2d. edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

The nucleic acid encoding the chimeric polypeptide can be any nucleic acid that functionally encodes the chimeric polypeptide. To functionally encode the polypeptide (i.e., allow the nucleic acid to be expressed), the nucleic acid can include, for example, expression control sequences, such as an origin of replication, a promoter, an enhancer, and necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from metallothionine genes, actin genes, immunoglobulin genes, CMV, SV40, adenovirus, bovine papilloma virus, etc. A nucleic acid encoding a selected chimeric polypeptide can readily be determined based upon the genetic code for the amino acid sequence of the selected chimeric polypeptide and many nucleic acids will encode any selected chimeric polypeptide. Modifications in the nucleic acid sequence encoding the chimeric polypeptide are also contemplated. Modifications that can be useful are modifications to the sequences controlling expression of the chimeric polypeptide to make production of the chimeric polypeptide inducible or repressible as controlled by the appropriate inducer or repressor. Such means are standard in the art (*see, e.g.*,. Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989). The nucleic acids can be generated by means

standard in the art, such as by recombinant nucleic acid techniques, as exemplified in the examples herein, and by synthetic nucleic acid synthesis or *in vitro* enzymatic synthesis.

A vector comprising the nucleic acids of the present invention and a cell comprising the vector of the present invention is also provided. The vectors of the invention can be in a host (e.g., cell line or transgenic animal) that can express the chimeric polypeptide contemplated by the present invention.

There are numerous *E. coli* (*Escherichia coli*) expression vectors known to one of ordinary skill in the art useful for the expression of proteins. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteria, such as *Salmonella, Serratia*, as well as various *Pseudomonas* species. These prokaryotic hosts can support expression vectors which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (Trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence and have ribosome binding site sequences for example, for initiating and completing transcription and translation. If necessary, an amino terminal methionine can be provided by insertion of a Met codon 5' and in-frame with the protein. Also, the carboxy-terminal extension of the protein can be removed using standard oligonucleotide mutagenesis procedures.

Additionally, yeast expression can be used. There are several advantages to yeast expression systems. First, evidence exists that proteins produced in a yeast secretion systems exhibit correct disulfide pairing. Second, post-translational glycosylation is efficiently carried out by yeast secretory systems. The *Saccharomyces cerevisiae* pre-pro-alpha-factor leader region (encoded by the *MFα-1* gene) is routinely used to direct protein secretion from yeast (Brake et al., 1984. "Alpha-factor-directed

synthesis and secretion of mature foreign proteins in *Saccharomyces cerevisiae*," *PNAS* 82:4642-4646.). The leader region of pre-pro-alpha-factor contains a signal peptide and a pro-segment which includes a recognition sequence for a yeast protease encoded by the *KEX2* gene. This enzyme cleaves the precursor protein on the carboxyl side of a Lys-Arg dipeptide cleavage-signal sequence. The polypeptide coding sequence can be fused in-frame to the pre-pro-alpha-factor leader region. This construct is then put under the control of a strong transcription promoter, such as the alcohol dehydrogenase I promoter or a glycolytic promoter. The protein coding sequence is followed by a translation termination codon which is followed by transcription termination signals. Alternatively, the polypeptide coding sequence of interest can be fused to a second protein coding sequence, such as Sj26 or β-galactosidase, used to facilitate purification of the fusion protein by affinity chromatography. The insertion of protease cleavage sites to separate the components of the fusion protein is applicable to constructs used for expression in yeast.

Efficient post-translational glycosylation and expression of recombinant proteins can also be achieved in *Baculovirus* systems in insect cells.

Mammalian cells permit the expression of proteins in an environment that favors important post-translational modifications such as folding and cysteine pairing, addition of complex carbohydrate structures and secretion of active protein. Vectors useful for the expression of proteins in mammalian cells are characterized by insertion of the protein coding sequence between a strong viral promoter and a polyadenylation signal. The vectors can contain genes conferring either gentamicin or methotrexate resistance for use as selectable markers. The antigen and immunoreactive fragment coding sequence can be introduced into a Chinese hamster ovary (CHO) cell line using a methotrexate resistance-encoding vector. Presence of the vector RNA in transformed cells can be confirmed by Northern blot analysis and production of a cDNA or opposite strand RNA corresponding to the protein coding sequence can be confirmed by

Southern and Northern blot analysis, respectively. A number of other suitable host cell lines capable of secreting intact proteins have been developed in the art and include the CHO cell lines, HeLa cells, myeloma cell lines, Jurkat cells, and the like. Expression vectors for these cells can include expression control sequences, as described above. The vectors containing the nucleic acid sequences of interest can be transferred into the host cell by well-known methods, which vary depending on the type of cell host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cell hosts.

Alternative vectors for the expression of protein in mammalian cells, similar to those developed for the expression of human gamma-interferon, tissue plasminogen activator, clotting Factor VIII, hepatitis B virus surface antigen, protease Nexinl, and eosinophil major basic protein, can be employed. Further, the vector can include CMV promoter sequences and a polyadenylation signal available for expression of inserted nucleic acid in mammalian cells (such as COS7).

The nucleic acid sequences can be expressed in hosts after the sequences have been positioned to ensure the functioning of an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors can contain selection markers, e.g., tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those cells transformed with the desired nucleic acid sequences (*see*, e.g., U.S. Patent 4,704,362).

The present invention also provides a chimeric polypeptide comprising a HBcAg region and an antigenic polypeptide region comprising greater than 40 amino acids. Thus, the invention provides a chimeric polypeptide having an antigenic polypeptide region comprising, for example, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54,

55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299 or 300 amino acids. The present invention also contemplates a chimeric polypeptide comprising an HBcAg region and an antigenic polypeptide region comprising greater than 300 amino acids, which can function as a carrier for epitopes, in a conformation other than an assembled core particle of HBV. For example, the antigenic polypeptide region can comprise an immunoglobulin variable heavy chain, an immunoglobulin variable light chain, either of which can comprise about 100 to 130 amino acids, or the antigenic polypeptide region can comprise a single chain antibody which can comprise about 280 amino acids.

The chimeric polypeptide comprising an antigenic polypeptide region comprising greater than 40 amino acids can be in a composition comprising a pharmaceutically acceptable carrier and a suitable adjuvant, as described previously for the chimeric polypeptide comprising a tumor epitope. In addition, the present invention contemplates a nucleic acid encoding the chimeric polypeptide comprising a HBcAg region and an antigenic polypeptide region comprising greater than 40 amino acids, as well as a vector comprising the nucleic acid and a cell comprising the vector, as described previously.

The chimeric polypeptide comprising a HBcAg region and an antigenic polypeptide region can have the structure: HBcAg-X, wherein X is an antigenic polypeptide region comprising greater than 40 amino acids. Thus, the invention provides a chimeric polypeptide having an antigenic polypeptide region wherein X comprises, for example, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299 or 300 amino acids. For example, X can be an immunoglobulin variable heavy chain, an immunoglobulin variable light chain, either of which can comprise about 100 to 130 amino acids, or a single chain antibody, which can comprise about 280 amino acids. Also contemplated is a chimeric polypeptide comprising an epitope of a hepatitis B virus core antigen and an antigenic polypeptide region having the structure: HBcAg-X, wherein X is an antigenic polypeptide region comprising greater than 300 amino acids, which can assemble into core particles or can function as a carrier of various epitopes in a conformation which is not an assembled core particle.

A chimeric polypeptide comprising a HBcAg region and a antigenic polypeptide region comprising greater than 40 amino acids, which assembles into core particles, is also provide in the present invention. Thus, the invention provides a chimeric polypeptide having an antigenic polypeptide region comprising, for example, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299 or 300 amino acids, which can assemble into core particles. For example, the antigenic polypeptide region can comprise an immunoglobulin variable heavy chain, an immunoglobulin variable light chain, either of which can comprise about 100 to 130 amino acids, or the antigenic polypeptide region can comprise a single chain antibody, which can comprise about 280 amino acids and which can assemble into core particles. The present invention also contemplates a chimeric polypeptide comprising an HBcAg region and an antigenic polypeptide region comprising greater than 300 amino acids, which can assemble into core particles.

The chimeric polypeptide comprising an antigenic polypeptide region comprising greater than 40 amino acids, which can assemble into core particles can be in a

composition comprising a pharmaceutically acceptable carrier and a suitable adjuvant, as described previously for the chimeric polypeptide comprising a tumor epitope. In addition, the present invention contemplates a nucleic acid encoding the chimeric polypeptide comprising a HBcAg region and an antigenic polypeptide region comprising greater than 40 amino acids, which can assemble into 40 nm like particles, as well as a vector comprising the nucleic acid and a cell comprising the vector, as described previously.

The present invention also provides a method for inducing an immune response in a subject capable of inducing an immune response and preferably human, comprising administering to the subject an immune response inducing amount of a chimeric polypeptide comprising a HBcAg region and an antigenic polypeptide region comprising greater than 40 amino acids. As used herein, "an immune response inducing amount" is that amount of chimeric polypeptide which is capable of producing in a subject a humoral and/or cellular immune response capable of being detected by standard methods of measurement, such as, for example, as described herein. For example, the antigenic polypeptide region can induce an antibody response. The antibodies can treat or prevent a pathological or harmful condition in the subject in which the antibodies are produced or the antibodies can be removed from the subject and administered to another subject to treat or prevent a pathological or harmful condition.

In an embodiment wherein the antigenic polypeptide region comprises an immunoglobulin light or heavy chain or a single chain antibody, the immune response can be the production in the subject of anti-idiotype antibodies, which represent the image of the original antigen and can function in a vaccine preparation to induce an immune response to a pathogenic antigen, thereby avoiding immunization with the antigen itself (Harlow and Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1988). The anti-idiotype antibodies can treat or prevent a pathological or harmful condition in the subject in which the anti-

idiotype antibodies are produced or the anti-idiotype antibodies can be removed from the subject and administered to another subject to treat or prevent a pathological or harmful condition.

Further provided is a method for inhibiting the growth of tumor cells in a subject, preferably human, comprising administering to the subject a tumor cell growth-inhibiting amount of a chimeric polypeptide comprising a HBcAg region and an antigenic polypeptide region comprising greater than 40 amino acids.

Additionally, the chimeric polypeptide comprising an HBcAg region and an antigenic polypeptide region comprising greater than 40 amino acids can be used in *in vitro* diagnostic assays, as well as in screening assays for identifying unknown tumor epitopes and fine mapping of tumor epitopes.

Also provided is a method for producing a chimeric polypeptide comprising a HBcAg region and an antigenic polypeptide region comprising greater than 40 amino acids, which assembles into core particles, comprising cloning into an expression vector a first DNA fragment encoding a HBcAg region and a second DNA fragment encoding an antigenic polypeptide region greater than 40 amino acids; and expressing the DNA of the expression vector in an expression system under conditions whereby the chimeric polypeptide assembles into core particles. The expression vector and expression system can be of any of the types as described herein. The cloning of the first and second DNA segments into the expression vector and expression of the DNA under conditions which allow for the formation of core particles can be carried out as described in the Examples section included herein. As used herein, the phrase "assembles into core particles" means that the chimeric polypeptide self assembles by a mechanism similar to the self assembly mechanism of wild type virus core protein in mammalian cells.

In a further preferred embodiment, the present invention provides a method of inhibiting the growth of tumor cells in any animal subject capable of eliciting an immune response to a tumor, preferably human, comprising administering to the subject a tumor cell growth-inhibiting amount of a chimeric polypeptide comprising a HBcAg region and a region comprising an epitope of a tumor antigen corresponding to a tumor antigen in the subject. As used herein, "inhibiting the growth of tumor cells" means that following administration of the chimeric polypeptide, a measurable immune response against the tumor cell epitope is elicited in the subject, resulting in the inhibition of growth of tumor cells present in the subject. The humoral immune response can be measured by detection, in the serum of the subject, of antibodies reactive with the epitope of the tumor antigen present on the chimeric polypeptide, according to protocols standard in the art, such as enzyme linked immunosorbent immunoassay (ELISA) and Western blotting protocols. The cellular immune response can be measured by, for example, footpad swelling in laboratory animals, peripheral blood lymphocyte (PBL) proliferation assays and PBL cytotoxicity assays, as would be known to one of ordinary skill in the art of immunology and particularly as set forth in the available handbooks and texts of immunology protocols (see, for example," Immunologic Studies in Humans," In *Current Protocols in Immunology*, J. E. Coligan et al., eds. John Wiley & Sons, New York (1991).

To determine the effect of the administration of the chimeric polypeptide on inhibition of tumor cell growth in laboratory animals, the animals can either be pre-treated with the chimeric polypeptide and then challenged with a lethal dose of tumor cells, or the lethal dose of tumor cells can be administered to the animal prior to receipt of the chimeric polypeptide and survival times documented. To determine the effect of administration of the chimeric polypeptide on inhibition of tumor cell growth in humans, standard clinical response parameters can be analyzed. These parameters, as would be known to one of ordinary skill in the art of oncology and tumor biology, can include

physical examination of the subject, measurements of tumor size, X-ray studies and biopsies.

The chimeric polypeptide can be administered to the subject orally or parenterally, as for example, by intramuscular injection, by intraperitoneal injection, topically, transdermally, injection directly into the tumor, or the like, although subcutaneous injection is typically preferred. Immunogenic and tumor cell growth inhibiting amounts of the chimeric polypeptide can be determined using standard procedures, as described. Briefly, various doses of the chimeric polypeptide are prepared, administered to a subject and the immunological response to each dose is determined. (Arnon, R. (Ed.) *Synthetic Vaccines* I:83-92, CRC Press, Inc., Boca Raton, Florida, 1987). The exact dosage of the chimeric polypeptide will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the cancer that is being treated, the particular tumor antigen being used, the mode of administration, and the like. Thus, it is not possible to specify an exact amount. However, an appropriate amount may be determined by one of ordinary skill in the art using only routine screening given the teachings herein.

Generally, the dosage will approximate that which is typical for the administration of vaccines, and typically, the dosage will be in the range of about 1 to 500 μg of the chimeric polypeptide per dose, and preferably in the range of 50 to 250 μg of the chimeric polypeptide per dose. This amount can be administered to the subject once every other week for about eight weeks or once every other month for about six months. The effects of the administration of the chimeric polypeptide can be determined starting within the first month following the initial administration and continued thereafter at regular intervals, as needed, for an indefinite period of time.

Parenteral administration, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or

suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system, such that a constant level of dosage is maintained. See, e.g., U.S. Patent No. 3,710,795, which is incorporated by reference herein.

For solid compositions, conventional nontoxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talc, cellulose, glucose, sucrose, magnesium carbonate, and the like. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc. an active compound as described herein and optional pharmaceutical adjuvants in an excipient, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington's Pharmaceutical Sciences* (Martin, E.W. (ed.), latest edition, Mack Publishing Co., Easton, PA).

For oral administration, fine powders or granules may contain diluting, dispersing, and/or surface active agents, and may be presented in water or in a syrup, in capsules or sachets in the dry state, or in a nonaqueous solution or suspension wherein suspending agents may be included, in tablets wherein binders and lubricants may be included, or in a suspension in water or a syrup. Where desirable or necessary, flavoring, preserving, suspending, thickening, or emulsifying agents may be included. Tablets and granules are preferred oral administration forms, and these may be coated.

The present invention also provides a method for producing single chain antibodies against tumor epitopes comprising producing an chimeric polypeptide comprising a HBcAg region and a region comprising an epitope of a tumor antigen; immunizing animals with an amount of the chimeric polypeptide sufficient to produce a humoral immune response to the chimeric polypeptide; isolating spleen cells expressing immunoglobulin specific for the chimeric polypeptide; isolating the immunoglobulin variable genes from the spleen cells; cloning the immunoglobulin variable genes into an expression vector; expressing the immunoglobulin variable genes in a bacteriophage; infecting *E. coli* cells with the bacteriophage; isolating bacteriophage from the *E. coli* cells which express the immunoglobulin variable genes and isolating the immunoglobulin variable gene products for use as single chain antibodies.

For example, tumor epitopes in HBcAg particles would be repeated 180 to 240 times per core particle and substantial amounts of epitope would be exposed on the surface of the chimeric core particles. These particles, such as Muccore, would be better targets than tumor cells or purified tumor antigen peptides for antibody selection approaches such as phage displayed scFv production. For example, there are two ways to produce specific Fv displayed on the surface of phage: (1) Immunize mice with tumor cells; isolate immunoglobulin variable fragment genes from spleen cells by RT/PCR; clone the genes into bacteriophage in frame with genes coding phage surface proteins (e.g., major coat protein subunits gpVIII or gp III of the filamentous bacteriophage) (Willis et al., 1993. *Gene* **128**:79-83; Jellis et al., 1993. *Gene* **137**:63-68). (2) Construct semisynthetic antibody libraries by PCR as described (Barbas et al., 1992. PNAS 89:4457-4461). The specific phage producing scFv are selected by several rounds of binding elution and infection in *E. coli*, using biotin labeled HBcAg-tumor epitope particles (e.g., Muccore). The biotin enables selection of high affinity scFv-phage through binding to streptavidin conjugated magnetic beads. This approach provides simple, fast and efficient production of specific anti-tumor epitope scFv.

The present invention is more particularly described in the following examples which are intended as illustrative only as numerous modifications and variations therein will be apparent to those skilled in the art.

EXAMPLES

Example I. Construction of a chimeric polypeptide

Cloning of HBcAg. The gene coding for HBcAg (Schodel et al. 1989. *Adv. Viral Oncol.* 8:73-102) was amplified by the polymerase chain reaction (PCR) (30 cycles of 30 sec. at 94°C, followed by 30 sec. at 56°C, followed by 30 sec at 72°C) from the plasmid pHBV320, which contains the entire genome of hepatitis B virus (HBV) (subtype ayw, gift of Dr. P. Pumpens, Riga, Latvia), using primers PRHBc5'-1 (CCATGGACATTGATCCTTATAAAGAA) (SEQ ID NO:1) and PRHBc3'-R1 (AAGCTTAAGATCTTCTGCGACGCGG (SEQ ID NO:2). The HBcAg DNA fragment was cloned using Nco I and Hind III sites into the N1pBtaccore70'/AB expression vector. The expression vector N1pBtaccore70'/AB was constructed by inserting a PCR fragment with a Shine Delgarno sequence, optimized 5'-UTR, a portion of the amino-terminus of HBcAg (70 bp) and unique restriction enzyme sites into the pBtac2 plasmid (Boehringer Mannheim).

A tumor epitope was inserted into HBcAg either through insertion between positions 78 and 79 in the amino acid sequence or insertion after either position 149 or 176 of the amino acid sequence near the carboxy terminus of HBcAg. To simplify the cloning of tumor epitopes, unique restriction sites (Xho I and Sma I) were incorporated between the nucleotide positions in the HBcAg gene corresponding to positions 78 and 79 in the amino acid sequence.

Cloning of the MUC1 epitope. A 20-mer epitope of MUC1: PDTRPAPGSTAPPAHGVTSA (SEQ ID NO: 3) was PCR-amplified from the

oligonucleotide GGATCCGGACACTCGCCCAGCACCGGGTTCTACTGCTCCGCCGGCA CACGGTGTAACTTCTGCCCGGG (SEQ ID NO:4) using primers PRMUC-1 (GGATCCGGACACTCGCCCA) (SEQ ID NO:5) and PRMUC-R1 (CCCGGGCAGAAGTTACACCGTG (SEQ ID NO:6) and cloned into the HBcAg gene utilizing Mbo I and Sma I sites incorporated between the nucleotide positions in the HBcAg gene corresponding to positions 78 and 79 in the amino acid sequence.

In a second HBcAg-mucin fusion construct, a modified MUC1 epitope GVTSAPDTRPAPGSTAPPAH (SEQ ID NO:7) containing the immunogenic sequence PDTRP (SEQ ID NO:8), located internally, was used. This modified MUC1 epitope was PCR-amplified using primers PRMUC1M-5' (CTCGAGGGTGTAACTTCTGCCCCGGACACTCGCCCAGCA) (SEQ ID NO:9) and PRMUC1M-3' (CCCGGGCGTGTGCCGGCGGAGC) (SEQ ID NO:10) and cloned into the HBcAg gene sequence using Xho I and Sma I sites incorporated between the nucleotide positions in the HBcAg gene corresponding to positions 78 and 79 in the amino acid sequence. The resulting fusion proteins containing HBcAg and MUC1 epitopes were called Muccore.

Cloning of fragments from mutated Ha-ras oncogene. The ras fragments were amplified from the oligonucleotide Olig-RAS12-1 (AAGCTTGTGGTGGTGGGCGCAGGAGGCGTCGGAAAGAGTGCCCTGACCAT CCAGCTG) (SEQ ID NO:11) and OligRAS61-1 (ACCGCAGGTCAGGAAGAGTACTCTGCTATGCGCGACCAGTAC) (SEQ ID NO:12), corresponding to the regions of Ha-ras from positions 5 to 23 and 58 to 71 in the amino acid sequence, respectively.

(a) Cloning between the nucleotide positions in the HBcAg gene corresponding to amino acids at positions 78 and 79.

A DNA fragment corresponding to amino acids 5 to 23 of the Ha-ras oncogene product containing an Arg-12 mutation was PCR-amplified using primers PRRAS12M-1 (GTCGACAAGCTTGTGGTGGTGGGCGCAAGAGGC) (SEQ ID NO:13) and PRRASW-R1 (CCCGGGTCAGCTGGATGGTCAGGGCACT) (SEQ ID NO:14) and cloned into the HBcAg gene. A DNA fragment corresponding to amino acids 58 to 71 of the ras oncogene product containing a Leu-61 mutation was PCR-amplified using primers PRRAS61L-1 (CTCGAGATCCTGGACACCGC GGTCTGGAAGAG) (SEQ ID NO:15) and PRRAS61WT-R2 (CCCGGGCAGACATGTACTGGTCGCGCA) (SEQ ID NO:16) and cloned into the HBcAg gene. The carboxy-terminal portion of HBcAg was truncated up to position 149 in the amino acid sequence in all the above constructs to remove the poly-Arg region of HBcAg.

(b) Cloning into the HBcAg gene site corresponding to the position following amino acid 176, near the carboxy terminus.

Two types of PCR-generated Ha-ras fragments containing an Arg-12 mutation, corresponding to modified fragments with and without amino-terminal valine residues, were PCR-amplified using primer pairs PRRAS12M-1 (SEQ ID NO:13)/PRRASW-R1 (SEQ ID NO:14) and PRRAS12RT-2 (CTCGAGGCCGGCGGCGCAAGAGGCGTGGGA) (SEQ ID NO:17)/PRRASW-R1 (SEQ ID NO:14) and cloned, through intermediate vectors, into the nucleotide position in the HBcAg gene corresponding to the site after position 176 at the carboxy terminus in the HBcAg amino acid sequence. The final expression vectors also contained the nucleotide sequences of *c-myc* and a six amino acid histidine tag, fused in frame to the carboxy terminus of Ha-ras to facilitate detection and purification of the chimeric particles.

Cloning of fragments from other tumor antigens. DNA representing epitopes from other tumor antigens can be isolated or synthesized by methods known in the art, as described above, amplified by PCR and cloned into a construct containing all or a

portion of the HBcAg gene. These amplified DNA sequences can be inserted internally into the nucleotide sequence of the HBcAg gene or at either the carboxy terminus or amino terminus of the HBcAg gene. The DNA can be inserted either by splicing into restriction sites that are created or occur naturally in the HBcAg gene sequence or the DNA can be ligated at the desired insertion site with DNA ligase, for example, on the end of an oligonucleotide. The chimeric construct can be inserted into an expression vector by standard cloning techniques, e.g., as described above. All of these procedures are standard molecular biology techniques (as set forth, for example, in Sambrook et al., 1989. *Molecular Cloning: A Laboratory Manual*, 2d. edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and would be well known to one of ordinary skill in the art of molecular biology.

Construction of the chimeric HBcAg fused to the lymphoma specific variable fragments of immunoglobulin molecule or scFv. To express HBcAg fused with immunoglobulin variable heavy or light chain fragments, a plasmid was constructed consisting of three units: (1) DNA fragment (fragment-1, core fragment) coding for HBcAg (from amino acid residues 1 to 176); (2) fragment-2, coding for the entire portion of either heavy or light variable chains of murine lymphoma 38c13 specific immunoglobulin; and (3) an expression vector for *E.coli*, such as the two commercially available vectors with pTac or T7 promoters, pBTac2 (Boehringer Mannhein, Indianapolis, IN) and pET11d (Stratagene, La Jolla, CA) plasmids, respectively. Both vectors were modified to introduce unique restriction sites and the sequence coding for c-myc tag and six His residues:

GGATCCGCAGAAGAACAGAAACTGATCTCAGAAGAGGATCTGGCCCACCAC

CATCACCATCACTAACCCGGG (SEQ ID NO:18), where Bam HI and Sma I sites are underlined and the ochre stop codon is marked in bold letters.

The core fragment was amplified by PCR (30 cycles of 30 seconds each at 94, 58 and 72°C) from the plasmid containing the entire HBV genome, subtype ayw (Dr.

Pauls Pumpa, Riga, Latvia) using specific oligonucleotide primers: PRCORE5'-1: GAATTCGGAGGAAAAAAACCATGGACATTGATCCTTATAAAGAA (SEQ ID NO:19) (Eco RI and Nco I sites underlined and SD and ATG codon in bold); PRCORE3'R1: AGATCTTCTGCGACGCGG (SEQ ID NO:20) (Bgl II site underlined). The fragment-1, purified from an agarose gel, was digested with Bgl II restriction endonuclease in the manufacturer's recommended buffer containing 50 mM Na-salt (Life Technologies, Gaithersburg, MD) for one hour at 37°C. The fragment ends were filled with dNTP (100 mM) using two u of Klenow fragment for ten minutes at room temperature (RT). The fragment was extracted with phenol-chloroform and precipitated in ethanol containing 0.3 M Na-acetate. The fragment-1 was digested with Eco RI restriction endonuclease and extracted and precipitated as described. The resulting fragment was resuspended in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

The fragment-2 (Vh), coding for the variable heavy chain from the murine lymphoma 38c13 immunoglobulin, was removed similarly from the plasmid Vh38G4S pCR2.1/AB (Arya Biragyn) using Nco I and Bam HI endonuclease digestions. The Nco I end of the fragment was filled, as described above.

To clone fragment-1 and fragment-2 into the pBTac based vector, the plasmid N3scFv38MHpBtac/AB (Arya Biragyn) was digested with Eco RI and Bam HI endonucleases. About 50 ng of the vector was mixed with 200 ng each of the fragments and ligated for 1-2 hours at 16°C in the ligation mixture containing 2mM ATP and 1-5 u of T4 DNA ligase. XL1 blue competent *E coli*. cells were transformed with 3 µl of the ligation mixture as described (Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Publishing), plated onto agar plates with 100 µg/ml ampicillin (Amp) and incubated overnight at 37°C. The colonies were screened by PCR using specific primers (described above) to HBcAg. The positive clones were verified by dideoxy sequencing (Dideoxi-sequencing kit, Amersham). The resulting plasmid was called N17coreL-Vh38MHpTac/AB. The expression vector N28coreL-

Vh38MHpET11/AB was constructed similarly by cloning fragment-1 and fragment-2 under T7 promoter control in the modified plasmid, pET11d/AB.

The DNA fragment for the variable light (Vl) chain of 38c13 IgM (Vk38) was removed from the plasmid N1scFv38CM/AB (Arya Biragyn) by Sal I and Bam HI digestion and inserted into the N28coreL-Vh38pET11/AB plasmid by replacing the Vh38 fragment, Xho I and Bam HI. The resulting plasmid was verified by DNA sequencing analysis and called N5coreL-Vk38MHpET11/AB.

The DNA fragment for the 38c13 lymphoma specific single chain antibody (scFv38) was excised from N4scFv38MHpTac/AB plasmid (Arya Biragyn) and fused in frame to the 3' end of the core fragment. The resulting expression vector was called N12core-scFv38MHpTac/AB.

E.coli expression and purification of core fusion proteins. Cells were grown overnight in 150-200 ml Superbroth medium (SB, Digene Diagnostics Inc., Beltsville, MD) containing 150-200 µg/ml Amp and 1% dextrose at 30°C, at a shaker speed of about 180-200 rpm. Cell cultures were diluted 2X in the same medium in a 2 L flask and grown for an additional two hours. The cell culture was then diluted with 1 L of SB containing 150 µg/ml AMP and 0.5 mM IPTG (powdered IPTG was added directly to the medium) and cells were incubated further at 30°C on the shaker at a speed of 200-250 rpm for six hours. Cells were pelleted by centrifuging at 4000 rpm for 15 minutes at 4°C and the pellet was resupended in 20 ml Core lysis buffer (50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 100 mM NaCl), with 800 µl of lysozyme (50 mg/ml), 60 µl of 0.1 M PMSF and 140 µl of 150X proteinase inhibitor cocktail. The mixture was incubated on ice for 20 minutes. The cells were disrupted with three cycles of freeze-thawing. The bacterial DNA was digested for ten minutes at RT by adding 3 µl of DNase I (100,00 u/ml, Life Technologies, Gaithersburg, MD) and 200 µl of 1M $MgCl_2$. The cell debris was removed by centrifuging at 14,000 rpm for 20 minutes at 4°C (Sorvall RC-5B, rotor

SS-34). Recombinant HBcAg (core) was precipitated in 30% saturated ammonium sulfate (SAS). The pellet was respuspended in 7 ml of distilled water and dialyzed against water for one hour at 4°C in a dialysis membrane with a 50,000 KDa cut-off (Spectrum Medical Instruments Inc., Laguna Hills, CA). The core-V proteins were enriched by Hydroxy-apatite column chromatography (Bio-Rad Labs, Hercules, CA). The 0.2 M Na-phosphate fractions were pooled and precipitated with an equal volume of SAS. The pellet was resuspended in 3 ml of PBS containing 0.3% Triton X-100, 5% glycerol and the mixture was applied onto a C16/100 column with Sepharose 4CLB (Pharmacia Biotech, Uppsala, Sweden). The recombinant HBcAg particles came out in the fractions corresponding to the first pick just after the void volume. HBcAg positive fractions were pooled together and precipitated by adding equal volumes of SAS. The precipitated pellet of HBcAg particles was resuspended in 2 ml PBS and dialyzed against 4 L of PBS at 4°C overnight in 50,000 kDa cut-off dialysis membrane. The protein concentration was measured by Bradford assay (Pierce, Rockford, IL) with a BSA standard. The purity and quality of the particles were examined by PAGE, Western blot and ELISA as described (Current Protocols in Immunology, 1995, Coligan et al., eds. National Institutes of Health, John Wiley and Sons, Inc.) The samples were visualized by electron microscopy with negative staining.

Expression of core particles fused to the single chain antibody. The N12core-scFv38MHpTac/AB plasmid, encoding HBcAg (176 amino acid residues) fused to a single chain antibody fragment (scFv38, about 280 amino acid residues) was constructed to examine the size limits of the fusion protein on the particle formation ability of HBcAg. N12core-scFv38MHpTac/AB plasmid was expressed in the XL1 blue strain of *E. coli*. Western blot analysis with monoclonal antibodies to 9E10 (to c-myc tag; Sid Johnson, MedImmune, Gaithersburg, MD) or anti-WHBc (to denatured HBcAg, Florian Schoedel) demonstrated that bacterial lysates from IPTG induced cells contained the expected size band of 47 KDa. The best producer clone was used for purification of core-scFv38MH from the 1 L of batch culture. On the basis of data from ELISA assay

with Cl-5 monoclonal antibody (Vadin Bichko), which recognizes a conformational epitope on the surface of HBcAg particles, as well as electron microscopy, it was determined that the the fusion core-scFv38MH is folded into core particles (22-33 nm range in diameter). These data demonstrate that HBcAg can form particles when expressed as a fusion protein with a large polypeptide (e.g., scFv38).

Expression of core particles fused to the variable fragments of the immunoglobulin molecule from murine B cell lymphoma cells, 38c13. To increase the efficiency of particle formation of the core fusion proteins, experiments were carried out to express HBcAg fused to smaller fragments, such as entire portions of lymphoma specific immunoglobulin variable fragments. Two types of plasmids coding for HBcAg fused with Vh or Vk immunoglobulin fragments from B cell lymphoma 38c13 were constructed: N28coreL-Vh38MHpET11/AB and N5coreL-Vk38MHpET11/AB, respectively. The size of the immunoglobulin fragments fused to HBcAg (176 amino acid residues), including spacer regions and c-myc His-tags, was approximately 160 amino acids. A spacer sequence of about four glycine and one serine repeated three times, was introduced between the carboxy terminus of HBcAG and the amino end of the V fragments to enable efficient particle formation and surface exposure of V fragments. The plasmids were expressed in the BL21(DE3) strain of *E. coli* and the proteins produced were tested by Western blot hybridization using 9E10 and anti-WHBc antibodies (described above). Both antibodies detected a protein band of about 32-36 KDa. The highest producer clone was used for the purification of fusion proteins, core-Vh and core-Vk. The fusion core-Vh and core-Vk proteins migrated in Sepaharose 4CLB size exclusion chromatography very close to the void volume and were eluted from the first major pick, which corresponds to the partculated HBcAg. This fraction contained a positive band of the expected size for both the purified core-Vh and core-Vk samples with reducing PAGE. Non-reducing PAGE (14%) did not reveal a significant positive signal, which indicated that the majority of purified core-V proteins were assembled into particles. These data correlated well with the electron

micrographs of the samples, which showed that the fusion proteins of core-Vh and core-Vk were assembled into HBcAG-like particles. Sandwich ELISA of these samples revealed that variable fragments of the immunoglobulin molecule were exposed on the surface of the HBcAg particles. Thus, these data demonstrate that HBcAg retained the ability to efficiently self-assemble when expressed as a fusion protein with immunoglobulin variable region fragments.

HBcAg-fused immunoglobulin variable fragments could be used to recreate immunoglobulin idiotype on the surface of HBcAg particles, either by co-expressing both core-Vh and core-Vl in a single host, or by chemically induced particulation of denatured core-Vh and core-Vl proteins.

HBcAg particles expressing tumor epitopes for production of tumor epitope specific antibodies. These studies of HBcAg particles expressing Muccore indicate that the Muc-1 epitope is not only exposed on the surface of the HBcAg particle, but that it is also highly immunogenic in that environment. Anti-Muc-1 antibody response in both SCID and C57Bl/6 mice was readily produced when these animals were immunized with Muccore without adjuvants. The antibody response was amplified further when adjuvant was added. Thus, HBcAG particles bearing tumor epitopes are useful tools for generating anti-tumor epitope antibodies *in vivo*.

Tumor epitopes in HBcAg particles are repeated about 180 to 240 times per core particle, exposing substantial amounts of epitope on the surface of the chimeric core particles. These particles, like Muccore, are better targets than tumor cells or purified tumor antigen peptides for antibody selection protocols such as phage displayed scFv production. For example, the spleen cells from mice immunized with chimeric core particles (e.g., Muccore) are used for cloning of the immunoglobulin variable genes by RT/PCR protocols and subsequent expression on the surface of bacteriophage, using phage displayed scFv technology. The specific phage producing scFv is selected

through several rounds of binding, elution and *E. coli.* infection, using, for example, biotin-labeled Muccore particles and streptavidin-conjugated magnetic beads. This approach provides for fast, simple and efficient production of specific anti-tumor epitope scFv which bind tumor epitopes such as, for example, Muc-1 epitope.

Example II. *In vivo* studies on immunity and tumor suppression induced by administration of chimeric polypeptides.

Immunity and tumor suppression in hu-PBL-SCID mice against human pancreatic tumor cells. Two Muccore fusion proteins (differing by the position of the immunogenic sequence in the tumor antigen epitope, as described above), containing HBc antigen and a single tandem repeat of mucin antigen were constructed as described herein and studied as vaccines for inducing mucin-specific immune responses and tumor inhibition in hu-PBL-SCID mice. The hu-PBL-SCID mice received HBc-MUC1 as the active immunization. The vaccine was administered intraperitoneally at 60 μg/mouse for a total of three immunizations. The control animals received phosphate buffered saline as mock immunization. The anti-mucin antibody responses were measured by ELISA. The mucin-specific delayed type hypersensitivity was studied by determining mucin-induced footpad swelling and human lymphocytic infiltration. To study tumor inhibition, each animal received 5×10^6 mucin-expressing human pancreatic cancer (PANC-1) cells (Dr. Ming Lu, Department of Surgery, Brown University at Roger Williams Medical Center). The tumor growth kinetics were studied in both immunized and control animals. Both fusion proteins were shown to induce anti-mucin humoral and cellular immunities in hu-PBL-SCID mice. One preparation was shown to be effective in the induction of antibody response while the other one was found slightly more effective in inducing cellular immunity. Immunization with either preparation was shown to inhibit the growth of mucin-expressing human pancreatic cancer in hu-PBL-SCID mice. HBc-MUC1 fusion proteins were demonstrated to be immunogenic and tumor-suppressive in SCID mice reconstituted with human PBL.

Immunity and tumor suppression in BALB/c mice against lethal challenge with tumor cells harboring a Ha-ras Arg-12 mutation. A chimeric fusion HBcAg gene bearing a mutated amino-terminal portion of the Ha-ras oncogene was constructed as described herein and expressed in *E. coli*. The fusion protein, HBcAg/Arg-12 ras, efficiently self-assembles into core particles, as verified by electron microscopy. Results of immunogold electron microscopy analysis and ELISA using specific monoclonal antibody showed that the Arg-12-mutated Ha-ras fragment was exposed on the surface of HBcAg particles. To test the effect of the fusion protein on syngeneic tumor resistance, two groups of BALB/c mice were immunized with either 80 μg of purified HBcAg/Arg-12 ras or Muccore (as a control) and subsequently challenged with a lethal dose of Ha-Balb tumor cells containing arg-12-mutated ras (Dr. Larry Kwak, NCI, Frederick, MD). The control mice immunized with Muccore died by day 25 of post tumor challenge, with the median at day 20. However, HBcAg/Arg-12 immunized mice had significantly suppressed tumor growth and prolonged survival with median at day 35.

Example III. Administration of chimeric polypeptides to human subjects

Immunity and suppression of tumor growth in a human subject. To elicit a tumor cell growth inhibiting response in a human subject, a chimeric polypeptide comprising an epitope of HBcAg and an epitope of a tumor antigen present in the human subject is administered to the subject subcutaneously in a dose ranging from 1 to 500 μg of the polypeptide once weekly for about eight weeks or once monthly for about six months. Within the first month following the initial immunization, blood samples can be taken from the subject and analyzed to determine the effects of administration of the chimeric polypeptide. Particularly, the presence in the subject's serum, of antibodies reactive with the epitope of the tumor antigen on the chimeric protein can be determined by ELISA, Western blotting or radioimmunoprecipitation, or other methods for detecting the formation of antigen/antibody complexes as would be standard practice for one of ordinary skill in the art of immunology. Also, a cellular immune response to the

epitope of the tumor antigen in the chimeric polypeptide can be detected by PBL proliferation assays, PBL cytotoxicity assays, cytokine measurements, or other methods for detecting delayed type hypersensitivity and cellular immune response, as would be standard practice for one of ordinary skill in the art of immunology. Additionally, the kinetics of tumor growth and inhibition of tumor cell growth can be determined by monitoring the subject's clinical response, through physical examination, tumor measurement, x-ray analysis and biopsy. The exact dosage can be determined for a given subject by following the teachings as set forth herein, as would be standard practice for one of ordinary skill in the art of vaccine development.

Although the present invention has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the invention except as and to the extent that they are included in the accompanying claims.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties, as well as the references cited within these publications, are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The United States of America as represented by the Secretary, Department of Health and Human Services, c/o National Institutes of Health, Office of Technology Transfer
6022 Executive Boulevard, Suite 325
Rockville, Maryland 20852

(ii) TITLE OF INVENTION: METHODS AND COMPOSITIONS OF CHIMERIC POLYPEPTIDES FOR TUMOR ANTIGEN VACCINES

(iii) NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: NEEDLE & ROSENBERG, P.C.
(B) STREET: Suite 1200, 127 Peachtree Street
(C) CITY: Atlanta
(D) STATE: Georgia
(E) COUNTRY: USA
(F) ZIP: 30303

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: Corresponding to U.S.Ser. No. 60/013,839
(B) FILING DATE: 21-MAR-1996
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(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: MILLER, MARY L.
(B) REGISTRATION NUMBER: 39,303
(C) REFERENCE/DOCKET NUMBER: 14014.0186/P

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 404/688-0770
(B) TELEFAX: 404/688-9880

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCATGGACAT TGATCCTTAT AAAGAA 26

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AAGCTTAAGA TCTTCTGCGA CGCGG 25

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His Gly
1 5 10 15

Val Thr Ser Ala
20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGATCCGGAC ACTCGCCCAG CACCGGGTTC TACTGCTCCG CCGGCACACG GTGTAACTTC 60

TGCCCGGG 68

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGATCCGGAC ACTCGCCCA 19

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCCGGGCAGA AGTTACACCG TG 22

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala
1 5 10 15

Pro Pro Ala His
20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro Asp Thr Arg Pro
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTCGAGGGTG TAACTTCTGC CCCGGACACT CGCCCAGCA 39

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCCGGGCGTG TGCCGGCGGA GC 22

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AAGCTTGTGG TGGTGGGCGC AGGAGGCGTC GGAAAGAGTG CCCTGACCAT CCAGCTG 57

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ACCGCAGGTC AGGAAGAGTA CTCTGCTATG CGCGACCAGT AC 42

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTCGACAAGC TTGTGGTGGT GGGCGCAAGA GGC 33

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCCGGGTCAG CTGGATGGTC AGGGCACT 28

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTCGAGATCC TGGACACCGC GGTCTGGAAG AG 32

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCCGGGCAGA CATGTACTGG TCGCGCA 27

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTCGAGGCCG GCGGCGCAAG AGGCGTGGGA 30

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGATCCGCAG AAGAACAGAA ACTGATCTCA GAAGAGGATC TGGCCCACCA CCATCACCAT 60

CACTAACCCG GG 72

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GAATTCGGAG GAAAAAAACC ATGGACATTG ATCCTTATAA AGAA 44

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AGATCTTCTG CGACGCGG 18

What is claimed is:

1. A chimeric polypeptide comprising an a hepatitis B virus core antigen region and a region comprising an epitope of a tumor antigen.

2. The polypeptide of claim 1, wherein the epitope of the tumor antigen is from human epithelial cell mucin.

3. The polypeptide of claim 1, wherein the epitope of the tumor antigen is from the Ha-ras oncogene product.

4. A composition comprising the polypeptide of claim 1 and a pharmaceutically acceptable carrier.

5. The composition of claim 4, further comprising a suitable adjuvant.

6. A nucleic acid encoding the chimeric polypeptide of claim 1.

7. A vector comprising the nucleic acid of claim 6.

8. A cell comprising the vector of claim 7.

9. A method of inhibiting the growth of tumor cells in a subject, comprising administering to the subject a tumor cell growth-inhibiting amount of a chimeric polypeptide comprising a hepatitis B virus core antigen region and a region comprising an epitope of a tumor antigen, corresponding to a tumor antigen in the subject.

10. The method of claim 9, wherein the epitope of the tumor antigen is from the Ha-ras oncogene product.

11. The method of claim 9, wherein the epitope of the tumor antigen is from human epithelial cell mucin.

12. The method of claim 9, wherein the administration is parenteral.

13. The method of claim 12, wherein the administration is subcutaneous.

14. The method of claim 9, wherein the subject is human.

15. The method of claim 9, further comprising administering a suitable adjuvant.

16. A chimeric polypeptide comprising a hepatitis B virus core antigen region and an antigenic polypeptide region comprising greater than 40 amino acids.

17. The chimeric polypeptide of claim 16, wherein the antigenic polypeptide region comprises an immunoglobulin variable heavy chain.

18. The chimeric polypeptide of claim 16, wherein the antigenic polypeptide region comprises an immunoglobulin variable light chain.

19. The chimeric polypeptide of claim 16, wherein the antigenic polypeptide region comprises a single chain antibody.

20. The chimeric polypeptide of claim 16, wherein the antigenic polypeptide region comprises up to 280 amino acids.

21. A composition comprising the chimeric polypeptide of claim 16 and pharmaceutically acceptable carrier.

22. The composition of claim 21, further comprising a suitable adjuvant.

23. A nucleic acid encoding the chimeric polypeptide of claim 16.

24. A vector comprising the nucleic acid of claim 23.

25. A cell comprising the vector of claim 24.

26. The chimeric polypeptide of claim 16, having the structure: HBcAg-X, wherein X is an antigenic polypeptide region comprising greater than 40 amino acids.

27. A chimeric polypeptide comprising a hepatitis B virus core antigen region and an antigenic polypeptide region comprising greater than 40 amino acids, which assembles into core particles.

28. A method of constructing a chimeric polypeptide comprising a hepatitis B virus core antigen region and an antigenic polypeptide region comprising greater than 40 amino acids, which assembles into core particles, comprising:

(a) cloning into an expression vector a first DNA fragment encoding a hepatitis B core antigen region and a second DNA fragment encoding an antigenic polypeptide region greater than 40 amino acids; and

(b) expressing the DNA of the expression vector in an expression system whereby the chimeric polypeptide assembles into core particles.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 97/04656

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N15/36 C12N15/48 C12N15/62 C12N5/10
C07K14/47 C07K14/82 C07K14/02 A61K38/17 A61K39/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SECOND INTERNATIONAL CONFERENCE ON ENGINEERED VACCINES OF CANCER AND AIDS, SAN FRANCISCO, CALIFORNIA, USA, MARCH 3-5, 1995. CANCER BIOTHERAPY 10 (1). 1995. 85. ISSN: 1062-8401, XP002035977 BIRAGYN A ET AL: "E. coli expressed lymphoma Ig idiotype antigen fusion proteins and chimeric HBcAg particles bearing tumor epitopes." see the whole document --- -/--	1-28

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 1997	08.08.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hornig, H

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INTERNATIONAL SEARCH REPORT

Intern nal Application No
PCT/US 97/04656

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VACCINE, vol. 13, no. 13, - 1995 BUTTERWORTH-HEINEMANN LTD, BOSTON, MA, USA, pages 1152-1160, XP002035978 N.R. BOULTER ET AL.: "Theileria annulata sporozoite antigen fused to hepatitis B core antigen used in a vaccination trial"	16, 20-25, 27,28
Y	see page 1153, left-hand column, paragraph 2 see page 1158, left-hand column, line 57 - line 61	1,2,4-9, 11,15, 21,22

X	J. BIOTECHNOLOGY, vol. 44, no. 1-3, 26 January 1996, ELSEVIER, AMSTERDAM,NL, pages 91-96, XP002035979 F. SCHÖDEL ET AL.: "Hybrid hepatitis B virus core antigen as a vaccine carrier moiety: I. Presentation of foreign epitopes"	16,20-28
Y	see page 91, line 5 - line 7 see page 93, left-hand column, line 37 - line 39	1,2,4-9, 11-15, 21,22

X	FEBS LETTERS, vol. 259, no. 1, December 1989, ELSEVIER, AMSTERDAM, NL, pages 121-124, XP002035980 G.P. BORISOVA ET AL.: "Recombinant core particles of hepatitits B virus exposing foreign antigenic determinants on their surface"	16,20, 23-25, 27,28
Y	see the whole document	1,2,4-9, 11-15, 21,22

X	EP 0 421 635 A (WELLCOME FOUND) 10 April 1991	16,20, 23-28
Y	see page 3, line 45 - line 49 see page 3, line 54 - page 4, line 10	1,2,4-9, 11-15, 21,22

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INTERNATIONAL SEARCH REPORT

Intern· ·al Application No
PCT/US 97/04656

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 119, no. 1, 5 July 1993 Columbus, Ohio, US; abstract no. 2495q, RAJNER, ULRIKH ET AL: "Plasmid pHIV24-5, encoding a fusion protein of hepatitis B virus core antigen and envelope protein p24 of human immunodeficiency virus for expression in Escherichia coli" page 265; column 1; XP002035987 see abstract & SU 1 751 209 A (INST ORCH SINTEZA AN LATVSSR, USSR;OTDEL MEDITSINY UNIV GUMB) ---	16,20, 23-28
X	CHEMICAL ABSTRACTS, vol. 115, no. 3, 22 July 1991 Columbus, Ohio, US; abstract no. 27294d, ULRICH, R. ET AL: "Exposure of major immunodominant epitope of bovine leukemia virus envelope protein gp51 on the surface of hepatitis B core antigen capsids" page 592; column 1; XP002035988 see abstract & MOL. BIOL. (MOSCOW) (1991), 25(2), 368-74 CODEN: MOBIBO;ISSN: 0026-8984, 1991, ---	16,20, 23-25, 27,28
Y	VIROLOGY, vol. 200, no. 2, 1 May 1994, ACADEMIC PRESS, INC.,NEW YORK, US, pages 547-557, XP002035981 R.W. TINDLE ET AL.: "Chimeric hepatitis B core antigen particles containing B- and Th-epitopes of human papillomavirus type 16 E7 protein induce specific antibody and T-helper pesponses in immunised mice" cited in the application see the whole document ---	1,2,4-9, 11-15, 20-28
Y	NATURE, vol. 330, 26 November 1987, MACMILLAN JOURNALS LTD., LONDON,UK, pages 381-384, XP002035982 B.E. CLARKE ET AL.: "Improved immunogenicity of a peptide epitope after fusion to hepatitis B core protein" see the whole document --- -/--	1,2,4-9, 11-15, 20-28

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INTERNATIONAL SEARCH REPORT

Interna[illegible]al Application No
PCT/US 97/04656

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CANCER LETTERS, vol. 70, 1993, ELSEVIER SCIENTIFIC PUBLISHERS IRELAND LTD., IE, pages 143-150, XP002035983 G. DENTON ET AL.: "Induction of antibody responses to breast carcinoma associated mucins using synthetic peptide constructs as immunogens" see the whole document ---	1,2,4-9, 11-15, 20-28
Y	CANCER IMMUNOL. IMMUNTHER., vol. 36, 1993, SPRINGER VERLAG, BERLIN, BRD, pages 9-17, XP002035984 L. DING ET AL.: "Immunogenicity of synthetic peptides related to the core peptide sequence encoded by the human MUC1 mucin gene: effect of immunization on the growth of murine mammary adenocarcinoma cells transfected with the human MUC1 gene" see the whole document ---	1,2,4-9, 11-15, 20-28
Y	WO 90 05142 A (IMP CANCER RES TECH) 17 May 1990 see the whole document ---	1,2,4-9, 11-15, 20-28
Y	WO 95 03825 A (FINN OLIVERA J ;FONTENOT J DARRELL (US); MONTELARO RONALD C (US)) 9 February 1995 see the whole document ---	1,2,4-9, 11-15, 20-28
Y	WO 95 27505 A (BIOMIRA INC ;LONGENECKER B MICHAEL (CA); DING LEI (CA); REDDISH MA) 19 October 1995 see the whole document ---	1,2,4-9, 11-15, 20-28
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INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/US 97/04656

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. VIROLOGY, vol. 67, no. 6, June 1993, AM.SOC.MICROBIOL.,WASHINGTON,US, pages 3696-3701, XP002035986 G. BORISOVA ET AL.: "Hybrid hepatitis B virus nucleocapsid bearing an immunodominant region from hepatitis B virus surface antigen" cited in the application see the whole document	1-28
A	EP 0 385 610 A (WELLCOME FOUND) 5 September 1990 see the whole document	1-28
A	EP 0 271 302 A (SCRIPPS CLINIC RES) 15 June 1988 see the whole document	1-28
A	WO 91 03255 A (WELLCOME FOUND) 21 March 1991 see the whole document	1-28

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

:rnational application No.
PCT/US 97/04656

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 9-15
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

.ormation on patent family members

Interr· ·nal Application No
PC1/US 97/04656

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